510 09 1911,132

L Number	Hits	Search Text	DB	Time stamp
1	235	(((multiple or several or different or many or independent) adj (transformant or transformation)) same (resistance or selection)) and ((high or increased or	USPAT; US-PGPUB; EPO; JPO; DERWENT;	2003/08/27 07:49
2	349	efficient) adj3 (express or expression))	IBM_TDB USPAT; US-PGPUB; EPO; JPO; DERWENT;	2003/08/27 07:52
3	42	efficient) adj3 (express or expression)) (alkaline adj phosphatase) same (express or expression) and (((multiple or several or different or many or independent) adj3 (transformant or transformation)) same (resistance or selection)) and ((high or increased or efficient) adj3 (express or expression))) and yeast	IBM_TDB USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/08/27 07:53
4	86		USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/08/27 07:52
5	8	(alkaline adj phosphatase) same (express or expression) and (((multiple or several or different or many or independent) adj3 (transformant or transformation)) same (resistance or selection)) and ((high or increased or efficient) adj3 (express or expression) same yeast))	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/08/27 07:55
6	141		USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM TDB	2003/08/27 07:57
7	33	(transformation or transformants) adj10 alkaline adj phosphatase	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM TDB	2003/08/27 07:56
8	1	(transformation or transformants) adj10 alkaline adj phosphatase same yeast	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM TDB	2003/08/27 07:56
9	27	(transformation or transformants) adj10 alkaline adj phosphatase and yeast	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM TDB	2003/08/27 07:56
10	24	((transformation or transformants) same yeast same alkaline adj phosphatase) and (express or expression) adj20 alkaline adj phosphatase	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM TDB	2003/08/27 07:58
11	8	eukaryotic adj3 alkaline adj phosphatase	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM TDB	2003/08/27 07:58
12	173	(bovine or eukaryotic) adj5 alkaline adj phosphatase	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM TDB	2003/08/27 07:59

13	7	(bovine or eukaryotic) adj5 alkaline adj phosphatase same yeast	USPAT; US-PGPUB; EPO; JPO;	2003/08/27 08:00
			DERWENT; IBM_TDB	
14	3833	yeast adj10 expression adj system	USPAT; US-PGPUB; EPO; JPO; DERWENT;	2003/08/27 08:01
15	0	yeast adj10 expression adj system and (mulitple adj transformation)	IBM_TDB USPAT; US-PGPUB;	2003/08/27 08:01
			EPO; JPO; DERWENT; IBM_TDB	
16	6	yeast adj10 expression adj system and (multiple adj transformation)	USPAT; US-PGPUB; EPO; JPO; DERWENT;	2003/08/27
17	535	yeast adj10 expression adj system and multiple adj (transformation or copy or copies)	IBM_TDB USPAT; US-PGPUB; EPO; JPO; DERWENT;	2003/08/27 08:04
18	77	(yeast adj10 expression adj system and multiple adj (transformation or copy or copies)) and eukaryotic adj5 gene	IBM_TDB USPAT; US-PGPUB; EPO; JPO; DERWENT;	2003/08/27 08:04
19	71	eukaryotic same yeast adj10 expression same multiple adj (transformation or copy or copies)	IBM_TDB USPAT; US-PGPUB; EPO; JPO; DERWENT;	2003/08/27 08:09
20	67	eukaryotic adj5 gene same yeast adj10 expression same multiple adj (transformation or copy or copies)	IBM_TDB USPAT; US-PGPUB; EPO; JPO; DERWENT;	2003/08/27 08:05
21	4	(eukaryotic same yeast adj10 expression same multiple adj (transformation or copy or copies)) not (eukaryotic adj5 gene same yeast adj10 expression same multiple	IBM_TDB USPAT; US-PGPUB; EPO; JPO; DERWENT;	2003/08/27 08:08
22	1	adj (transformation or copy or copies)) producing adj10 eukaryotic same yeast same multiple adj (transformation or copy or copies)	IBM_TDB USPAT; US-PGPUB; EPO; JPO; DERWENT;	2003/08/27 08:10
23	4103	yeast same multiple adj3transformations	IBM_TDB USPAT; US-PGPUB; EPO; JPO; DERWENT;	2003/08/27 08:10
24	24	yeast same multiple adj3 transformations	IBM_TDB USPAT; US-PGPUB; EPO; JPO; DERWENT;	2003/08/27 08:12
25	1771	method adj10 expression adj10 yeast	IBM_TDB USPAT; US-PGPUB; EPO; JPO;	2003/08/27 08:13
26	0	method adj10 expression adj10 yeast same gene adj copy	DERWENT; IBM_TDB USPAT; US-PGPUB; EPO; JPO;	2003/08/27 08:14
			DERWENT; IBM_TDB	

	,			
27	14124	method adj10 expression adj10 yeast and	USPAT;	2003/08/27
	\$	(increased or multiple or high) copy adj number	US-PGPUB; EPO; JPO;	08:15
			DERWENT;	
			IBM_TDB	
28	80	,	USPAT;	2003/08/27
		(increased or multiple or high) adj5	US-PGPUB;	08:15
		copy adj number	EPO; JPO; DERWENT;	
			IBM TDB	
29	73	method adj5 expression adj10 yeast and	USPAT;	2003/08/27
		(increased or multiple or high) adj5	US-PGPUB;	08:16
		copy adj number	EPO; JPO;	
			DERWENT;	
30	4	 (eukaryotic same yeast adj10 expression	IBM_TDB USPAT;	2003/08/27
30	-	same multiple adj (transformation or copy	US-PGPUB;	08:16
		or copies)) not (eukaryotic adj5 gene	EPO; JPO;	00.10
		same yeast adj10 expression same multiple	DERWENT;	
		adj (transformation or copy or copies))	IBM_TDB	
31	70	method adj5 expression adj10 yeast and	USPAT;	2003/08/27
		(increase or multiple or high) adj5 copy	US-PGPUB;	08:17
		adj number	EPO; JPO; DERWENT;	
	İ		IBM TDB	
32	70	(method adj5 expression adj10 yeast and	USPAT;	2003/08/27
		(increase or multiple or high) adj5 copy	US-PGPUB;	08:20
		adj number) not (eukaryotic adj5 gene	EPO; JPO;	
		same yeast adj10 expression same multiple	DERWENT;	
33	69	adj (transformation or copy or copies))	IBM_TDB	2002/09/27
33	69	((method adj5 expression adj10 yeast and (increase or multiple or high) adj5 copy	USPAT; US-PGPUB;	2003/08/27
		adj number) not (eukaryotic adj5 gene	EPO; JPO;	00.27
		same yeast adj10 expression same multiple	DERWENT;	
		adj (transformation or copy or copies)))	IBM_TDB	
		and (tranform or transformation or		
34	20	transforming)	TTGD T	2002/00/07
34	38	((method adj5 expression adj10 yeast and (increase or multiple or high) adj5 copy	USPAT; US-PGPUB;	2003/08/27
	-	adj number) not (eukaryotic adj5 gene	EPO; JPO;	00.22
		same yeast adj10 expression same multiple	DERWENT;	
		adj (transformation or copy or copies)))	IBM_TDB	
		and (tranform or transformation or		
35	1.0	transforming) same (marker)	HCD2m-	2002/00/07
35	10	(((method adj5 expression adj10 yeast and (increase or multiple or high) adj5 copy	USPAT; US-PGPUB;	2003/08/27
		adj number not (eukaryotic adj5 gene	EPO; JPO;	00.22
		same yeast adj10 expression same multiple	DERWENT;	
		adj (transformation or copy or copies)))	IBM_TDB	'
		and (tranform or transformation or	-	
		transforming) same (marker)) and alkaline		
36	8	adj phosphatase	USPAT;	2002/09/27
30		((method adj5 expression adj10 yeast and (increase or multiple or high) adj5 copy	USPAT; US-PGPUB;	2003/08/27
		adj number) not (eukaryotic adj5 gene	EPO; JPO;	00.20
		same yeast adj10 expression same multiple	DERWENT;	
		adj (transformation or copy or copies)))	IBM_TDB	
		and (transform or transformation or	-	
		transforming) same (two or second) adj10		
37	0	marker (transform or transformation or	USPAT;	2003/08/27
~ '		transforming) same (two or second) adj10	US-PGPUB;	08:29
		marker same (high or ncrease or increased	EPO; JPO;	
		or efficient) adj5 expression adj5 yeast	DERWENT;	
			IBM_TDB	
38	1	(transform or transformation or	USPAT;	2003/08/27
		transforming) same (two or second) adj10	US-PGPUB;	08:29
		marker same (high or increase or increased or efficient) adj5 expression	EPO; JPO; DERWENT;	
		adj5 yeast	IBM TDB	

39	13		USPAT;	2003/08/27
		transforming) same (two or second) adj10	US-PGPUB;	08:34
		marker and (high or increase or increased	EPO; JPO;	
		or efficient) adj5 expression adj5 yeast	DERWENT;	
			IBM TDB	
40	0	marker same increasing adj5 (drug or	USPAT;	2003/08/27
		concentration) same yeast same copy adj	US-PGPUB;	08:35
		number	EPO; JPO;	
			DERWENT;	
			IBM TDB	
41	15	marker same increasing adj5 (drug or	USPAT;	2003/08/27
		concentration) and yeast same copy adj	US-PGPUB;	08:38
		number	EPO; JPO;	00.50
		Tumber	DERWENT;	
			IBM TDB	1
42	3511	marker same (increasing adj5 (drug or	USPAT;	2003/08/27
74	3311	concentration or amplifiable and yeast	US-PGPUB;	08:39
		same copy adj number and alkaline adj	EPO; JPO;	00.39
		phosphatase or (two or second) adj marker		
		phosphatase of (two of second) adj marker	DERWENT;	
43	20		IBM_TDB	0000 (00 (07
43	38		USPAT;	2003/08/27
		concentration) or amplifiable) and yeast	US-PGPUB;	08:46
		same copy adj number and (alkaline adj	EPO; JPO;	
		phosphatase or (two or second) adj	DERWENT;	
		marker)	IBM_TDB	
47	0	method adj10 alkaline adj phosphatase	USPAT;	2003/08/27
		adj5 expression same eukaryotic	US-PGPUB;	08:47
			EPO; JPO;	
	-		DERWENT;	
			IBM_TDB	
48	3	Parme manualite day prioppriations days	USPAT;	2003/08/27
		expression same eukaryotic	US-PGPUB;	08:49
			EPO; JPO;	
			DERWENT;	
			IBM TDB	
49	1	(human or bovine) adj10 alkaline adj	USPĀT;	2003/08/27
		phosphatase adj5 expression same	US-PGPUB;	08:49
		eukaryotic	EPO; JPO;	
		_	DERWENT;	
			IBM TDB	
50	39	(human or bovine) adj10 alkaline adj	USPAT;	2003/08/27
		phosphatase adj5 expression	US-PGPUB;	08:50
		Frankrama and and antended	EPO; JPO;	
			DERWENT;	
			IBM TDB	
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FULL ESTIMATED COST

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=> s alkaline (A) phosphatase

100771 ALKALINE (A) PHOSPHATASE L1

=> s (bovine or hyman or eukaryotic) (5A) alkaline (A) phosphatase 479 (BOVINE OR HYMAN OR EUKARYOTIC) (5A) ALKALINE (A) PHOSPHATASE 1.2

=> s 12 and express or produce (S) yeast

3318 L2 AND EXPRESS OR PRODUCE (S) YEAST L3

=> s 12 and (express or produce (S) yeast)

0 L2 AND (EXPRESS OR PRODUCE (S) YEAST) L4

=> s (express or produce (S) yeast) and (amplifiable (A) marker)

4 (EXPRESS OR PRODUCE (S) YEAST) AND (AMPLIFIABLE (A) MARKER)

=> d ibib abs 1-4

ANSWER 1 OF 4 MEDLINE on STN ACCESSION NUMBER: 2002133906 MEDLINE PubMed ID: 11834126 DOCUMENT NUMBER: 21823394

TITLE: High-level expression of human thyroid-stimulating hormone

in Chinese hamster ovary cells by co-transfection of dicistronic expression vectors followed by a dual-marker

amplification strategy.

AUTHOR: Peroni Cibele N; Soares Carlos R J; Gimbo Elizabeth; Morganti Ligia; Ribela Maria Teresa C P; Bartolini Paolo

CORPORATE SOURCE: Biotechnology Department, National Nuclear Energy

Commission (IPEN-CNEN), Travessa R-400, Cidade Universitaria, 05508-900, Sao Paulo, SP, Brazil.

BIOTECHNOLOGY AND APPLIED BIOCHEMISTRY, (2002 Feb) 35 (Pt SOURCE:

1) 19-26.

Journal code: 8609465. ISSN: 0885-4513.

England: United Kingdom PUB. COUNTRY:

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200205

ENTRY DATE: Entered STN: 20020301

Last Updated on STN: 20020502 Entered Medline: 20020501

The utilization of dicistronic mRNA expression vectors, containing the gene of interest upstream of an amplifiable marker gene, has shown success in rapidly, efficiently and reproducibly obtaining stable cell lines that express high levels of the protein of interest. For this reason, human thyroid-stimulating hormone (hTSH), a heterodimeric glycoprotein composed of non-covalently linked alpha- and beta-subunits, was expressed in Chinese hamster ovary (CHO) cells using a system based on dicistronic expression vectors. These contained the genes of interest and the amplifiable gene markers dihydrofolate reductase (DHFR) and adenosine deaminase (ADA), separated by an internal ribosome entry site isolated from the encephalomyocarditis virus. After the cells (CHO-DHFR-) had been co-transfected with the expression vectors and submitted to gene amplification in culture medium containing stepwise increments of methotrexate, it was possible to isolate clones that presented a secretion level of up to 7.2+/-1.3 microg/10(6) cells per day, the highest ever reported for the expression of this glycoprotein hormone. A second treatment, involving the utilization of deoxycoformycin, directed to amplify the ADA marker gene, provided a clone with an additional 2-3-fold increase in hTSH secretion, reaching a secretion level of 17.8+/-7.6 microg/10(6) cells per day. Cell culture and hTSH production in a hollow-fibre bioreactor were set up in order to carry out a preliminary physico-chemical, immunological and biological characterization of this hormone in comparison with pituitary-extracted hTSH (from the National Institute of Diabetes and Digestive and Kidney Diseases) and the only recombinant hTSH now available (Thyrogen). The availability of recombinant hTSH is very important in the diagnosis and

therapy of thyroid carcinoma, via stimulation of radioiodine uptake.

L5 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER:

2002:354032 CAPLUS

DOCUMENT NUMBER: TITLE:

Expression constructs comprising multiple units of

promoter linked to exon and unpaired splice sequence

and uses for improved gene expression

INVENTOR (S): Harrington, John J.

PATENT ASSIGNEE(S):

USA

SOURCE: U.S. Pat. Appl. Publ., 43 pp., Cont. of U.S. Ser. No.

136:351379

414,369, abandoned.

CODEN: USXXCO

DOCUMENT TYPE: LANGUAGE:

Patent English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO. KIND DATE

APPLICATION NO. DATE

-----US 2002055172 A1 20020509

US 2000-729416 20001205

PRIORITY APPLN. INFO.:

------US 1999-414369 B1 19991007

The present invention is directed to improved methods for gene expression using genetic vectors with at least two units, each unit comprising multiple promoters operably linked to an exon and unpaired splice sequence. Multiple promoter/exon units, which produce multiple RNA transcripts, are used in nucleic acid constructs to provide increased expression of a desired nucleic acid sequence. The sequence is introduced into a vector by conventional cloning or is expressed from an endogenous sequence in the genome that is activated by the vector contg. the multiple promoters. The vectors can be used to express cDNA clones, genes encoded by genomic DNA or fragments, activate endogenous genes in

situ, and modify gene or protein of interest. ANSWER 3 OF 4 CAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER:

2002:201542 CAPLUS

DOCUMENT NUMBER:

136:354224

TITLE:

High-level expression of human thyroid-stimulating

hormone in Chinese hamster ovary cells by

co-transfection of dicistronic expression vectors

followed by a dual-marker amplification strategy Peroni, Cibele N.; Soares, Carlos R. J.; Gimbo,

Elizabeth; Morganti, Ligia; Ribela, Maria Teresa C.

P.; Bartolini, Paolo

CORPORATE SOURCE:

Biotechnology Department, National Nuclear Energy

SOURCE:

AUTHOR (S):

Commission (IPEN-CNEN), Sao Paulo, 05508-900, Brazil Biotechnology and Applied Biochemistry (2002), 35(1),

19-26

CODEN: BABIEC; ISSN: 0885-4513

PUBLISHER: Portland Press Ltd. Journal

LANGUAGE:

English

The utilization of dicistronic mRNA expression vectors, contg. the gene of interest upstream of an amplifiable marker gene, has shown success in rapidly, efficiently and reproducibly obtaining stable cell lines that express high levels of the protein of interest. For this reason, human TSH (hTSH), a heterodimeric glycoprotein composed of non-covalently linked .alpha.- and .beta.-subunits, was expressed in Chinese hamster ovary (CHO) cells using a system based on dicistronic expression vectors. These contained the genes of interest and the amplifiable gene markers dihydrofolate reductase (DHFR) and adenosine deaminase (ADA), sepd. by an internal ribosome entry site isolated from the encephalomyocarditis virus. After the cells (CHO-DHFR-) had been co-transfected with the expression vectors and submitted to gene amplification in culture medium contg. stepwise increments of methotrexate, it was possible to isolate clones that presented a secretion level of up to 7.2.+-.1.3 .mu.g/106 cells per day, the highest ever reported for the expression of this glycoprotein hormone. A second treatment, involving the utilization of deoxycoformycin, directed to amplify the ADA marker gene, provided a clone with an addnl. 2-3-fold increase in hTSH secretion, reaching a secretion level of 17.8.+-.7.6 .mu.g/106 cells per day. Cell culture and hTSH prodn. in a hollow-fiber bioreactor were set up in order to carry out a preliminary physico-chem., immunol. and biol. characterization of this hormone in comparison with pituitary-extd. hTSH (from the National Institute of Diabetes and Digestive and Kidney Diseases) and the only recombinant hTSH now available (Thyrogen). The availability of recombinant hTSH is very important in the diagnosis and therapy of thyroid carcinoma, via stimulation of radio-iodine uptake.

REFERENCE COUNT: THERE ARE 47 CITED REFERENCES AVAILABLE FOR THIS

RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

ANSWER 4 OF 4 CAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: DOCUMENT NUMBER:

1987:418844 CAPLUS

107:18844

TITLE:

Use of the Escherichia coli gene for asparagine synthetase as a selective marker in a shuttle vector capable of dominant transfection and amplification in

animal cells

AUTHOR (S):

Cartier, Mireille; Chang, Mildred W. M.; Stanners,

Clifford P.

CORPORATE SOURCE:

Cancer Cent., McGill Univ., Montreal, QC, H3G 1Y6,

SOURCE:

Molecular and Cellular Biology (1987), 7(5), 1623-8

CODEN: MCEBD4; ISSN: 0270-7306

DOCUMENT TYPE:

Journal

LANGUAGE:

English

A new dominant amplifiable selective system for use in bacterium-animal cell shuttle vectors was developed by the insertion of a 2-kilobase genomic fragment contq. the cloned E. coli gene for asparagine synthetase (AS) into the pBR322-simian virus 40 recombinant vector pSV2 so as to place the translational initiator codon for the bacterial AS about 1,000 base pairs downstream from the simian virus 40 early promoter. This new construct, pSV2-AS, retains bacterial sequences for transcriptional and translational initiation and so can express AS in bacteria. The construct can also complement AS- mutants of mammalian cells, giving AS+ transfectants capable of growth in medium lacking asparagine, with relatively high efficiency (about 300 colonies per .mu.g of DNA per 106 cells exposed). The vector can be amplified up to 100-fold in such AS+ transfectants by selection in asparagine-free medium contg. increasing concns. of the AS inhibitor .beta.-aspartyl hydroxamate. AS+ transfectants were found to be much more resistant to a second AS inhibitor, Albizziin, than were normal AS+ animal cell lines. This difference, which may indicate a strong resistance of the bacterial AS enzyme to Albizziin, was exploited to develop an effective selection for bacterial AS transfectants of a no. of wild-type AS+ cell lines of rat, Chinese hamster, mouse, and human origin. LR-73 cells, a Chinese hamster AS+ cell line, were transfected with pSV2-AS with an efficiency of about 1,000 colonies per 0.5 .mu.g of DNA per 106 cells. The integrated construct in these cells was amplified by incubation of the transfectants in increasing concns. of .beta.-aspartyl hydroxamate. Advantages and disadvantages of this new dominant, selectable, and amplifiable marker over markers commonly used in shuttle vectors are discussed.

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=> s (express or produce (S) yeast) and (multiple (5A) marker)
           70 (EXPRESS OR PRODUCE (S) YEAST) AND (MULTIPLE (5A) MARKER)
L6
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- => s (express or produce (S) yeast) and (multiple (5A) marker) (S) increase (S) (copy or gene) 0 (EXPRESS OR PRODUCE (S) YEAST) AND (MULTIPLE (5A) MARKER) (S) INCREASE (S) (COPY OR GENE)
- => s (express or produce (S) yeast) and (multiple (5A) marker) and increase (S) (copy or gene) L80 (EXPRESS OR PRODUCE (S) YEAST) AND (MULTIPLE (5A) MARKER) AND INCREASE (S) (COPY OR GENE)